ABSTRACT

The ability to detect and quantify retinal ganglion cell (RGC) loss is important for the diagnosis and monitoring of optic neuropathies, such as glaucoma. This thesis describes the development, assessment, and implementation of intravitreal injection labelling of RGCs for non-invasive and longitudinal in vivo imaging in mice. We tested the hypothesis that the neuronal tracer, cholera toxin subunit B (CTB), and adeno-associated viral (AAV) vectors with a green fluorescent protein (GFP) reporter gene are detectable labels for RGCs. Following CTB labelling, individual cells were detected by in vivo imaging with confocal scanning laser ophthalmoscopy after 10-15 days and were successfully imaged consecutively up to 100-days post-injection. While it was found that CTB uptake was not restricted to RGCs, the majority of RGCs were labelled. These findings showed that intravitreal injection administration of CTB is a reliable and effective label for RGCs in mice by providing a clear, sustained and strong labelling of cells in the ganglion cell layer. AAV2-GFP labelling in retinal cells was detected by in vivo imaging at 1-week post-injection. An increase in the number of cells expressing GFP occurred until approximately week 4 post-injection. Immunohistochemistry showed that 5-weeks post-injection, the mean (standard error) GFP+ cells that were positive for the RGC-specific RBPMS marker was 86 (4)% for the AAV2-DCX-GFP (RGC specific) vector and 72 (3)% for the AAV2-CAG-GFP (non-RGC specific) vector. Functional responses of the retina were assessed with electroretinography. The positive and negative scotopic threshold responses, which measure RGC activity, had similar amplitudes between AAV2 injected and uninjected eyes. Both CTB- and AAV-based methods of intravitreal injection labelling provided strong and sustained fluorescence labelling in RGCs. However, AAV2 vectors demonstrated higher specificity to RGCs that could be further improved with the cell type-specific DCX promoter. This work demonstrates a technique for labelling and imaging the presence of healthy RGCs longitudinally, thereby providing a means to quantify changes in RGC density in experimental optic neuropathy, and after neuroprotective or neuroregenerative interventions.